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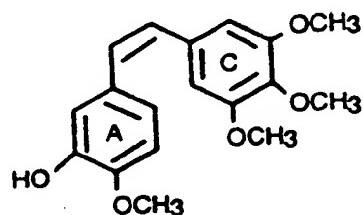
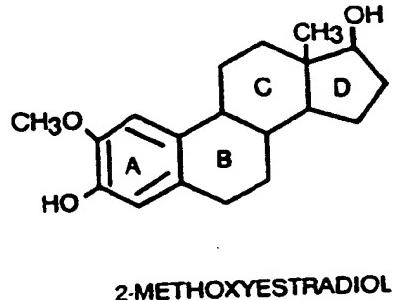
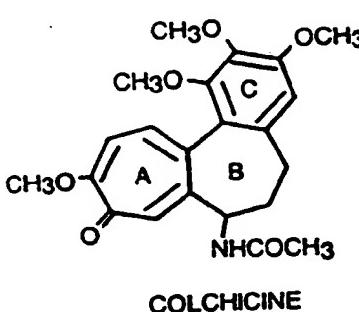
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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## (54) Title: ESTROGENIC COMPOUNDS AS ANTI-MITOTIC AGENTS



## (57) Abstract

The application discloses methods of making medicaments for treating mammalian diseases characterized by abnormal cell mitosis by administering estradiol derivatives including those comprising colchicine or combretastatin A-4 structural motifs of general formulae found above in a dosage sufficient to inhibit cell mitosis. The application discloses novel compounds used in the methods.

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ESTROGENIC COMPOUNDS  
AS ANTI-MITOTIC AGENTS

Background of the Invention

5 This invention relates to treating disease states characterized by abnormal cell mitosis.

Cell mitosis is a multi-step process that includes cell division and replication (Alberts, B. et al. In *The Cell*, pp. 652-661 (1989); Stryer, E.

10 *Biochemistry* (1988)). Mitosis is characterized by the intracellular movement and segregation of organelles, including mitotic spindles and chromosomes. Organelle movement and segregation are facilitated by the polymerization of the cell protein tubulin. Microtubules  
15 are formed from  $\alpha$  and  $\beta$  tubulin polymerization and the hydrolysis of GTP. Microtubule formation is important for cell mitosis, cell locomotion, and the movement of highly specialized cell structures such as cilia and flagella.

20 Microtubules are extremely labile structures that are sensitive to a variety of chemically unrelated anti-mitotic drugs. For example, colchicine and nocadazole are anti-mitotic drugs that bind tubulin and inhibit tubulin polymerization (Stryer, E. *Biochemistry* (1988)).

25 When used alone or in combination with other therapeutic drugs, colchicine may be used to treat cancer (WO-9303729-A, published March 4, 1993; J03240726-A, published October 28, 1991), alter neuromuscular function, change blood pressure, increase sensitivity to  
30 compounds affecting sympathetic neuron function, depress respiration, and relieve gout (*Physician's Desk Reference*, Vol. 47, p. 1487, (1993)).

Estradiol and estradiol metabolites such as 2-methoxyestradiol have been reported to inhibit cell division (Segers, J.C. et al. *J. Steroid Biochem.* 32,

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change (increase or decrease) in estrogen receptor binding, improved absorption, transport (e.g. through blood-brain barrier and cellular membranes), biological stability, or decreased toxicity. I have also discovered  
5 certain compounds useful in the method, as described by the general formulae of the claims.

A mammalian disease characterized by undesirable cell mitosis, as defined herein, includes but is not limited to excessive or abnormal stimulation of  
10 endothelial cells (e.g., atherosclerosis), solid tumors and tumor metastasis, benign tumors, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, vascular malfunctions, abnormal wound healing, inflammatory and immune disorders,  
15 Bechet's disease, gout or gouty arthritis, abnormal angiogenesis accompanying: rheumatoid arthritis, psoriasis, diabetic retinopathy, and other ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplastic), macular degeneration, corneal  
20 graft rejection, neovascular glaucoma and Osler Weber syndrome. Other undesired angiogenesis involves normal processes including ovulation and implantation of a blastula. Accordingly, the compositions described above can be used to block ovulation and implantation of a  
25 blastula or to block menstruation (induce amenorrhea).

The bond indicated by C••C is absent or, in combination with the C---C bond is the unit HC=CH.

Other features and advantages of the invention will be apparent from the following description of  
30 preferred embodiments thereof.

#### Description of the Preferred Embodiments

The drawings are first described.

Fig. 1 is a graph illustrating the inhibition of tubulin polymerization by 2-methoxyestradiol described by  
35 Example 1 below.

- 5 -

Figure 3, part I, depicts the chemical formulae of colchicine, 2-methoxyestradiol and combretastatin A-4. Figure 3, part IIa-d, illustrates estradiol derivatives that comprise structural motifs found in colchicine or 5 combretastatin A-4. For example, part II a-c shows estradiol derivatives with an A and/or B ring expanded from six to seven carbons as found in colchicine and part IIId depicts an estradiol derivative with a partial B ring as found in combretastatin A-4. Each C ring of an 10 estradiol derivative, including those shown in Figure 3, may be fully saturated as found in 2-methoxyestradiol. R<sub>1</sub>-6 represent a subset of the substitution groups found in the claims. Each R<sub>1</sub>-R<sub>6</sub> can independently be defined as -R<sub>1</sub>, OR<sub>1</sub>, -OCOR<sub>1</sub>, -SR<sub>1</sub>, -F, -NHR<sub>2</sub>, -Br, -I, or -C≡CH.

15 **Anti-mitotic Activity In Situ**

Anti-mitotic activity is evaluated *in situ* by testing the ability of an improved estradiol derivative to inhibit the proliferation of new blood vessel cells (angiogenesis). A suitable assay is the chick embryo 20 chorioallantoic membrane (CAM) assay described by Crum et al. *Science* 230:1375 (1985). See also, U.S. Patent 5,001,116, hereby incorporated by reference, which describes the CAM assay. Briefly, fertilized chick embryos are removed from their shell on day 3 or 4, and a 25 methylcellulose disc containing the drug is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear avascular zone appears around the methylcellulose disc, the diameter of that zone is measured. Using this assay, a 100mg disk of the 30 estradiol derivative 2-methoxyestradiol was found to inhibit cell mitosis and the growth of new blood vessels after 48 hours. This result indicates that the anti-mitotic action of 2-methoxyestradiol can inhibit cell mitosis and angiogenesis.

- 7 -

graft r jection, neurosular glacoma and Oster Webber syndrome.

Improved Estradiol Derivative Synthesis

Known compounds that are used in accordance with  
5 the invention and precursors to novel compounds according  
to the invention can be purchased, e.g., from Sigma  
Chemical Co., St. Louis, Steroloids and Research Plus.  
Other compounds according to the invention can be  
synthesized according to known methods from publicly  
10 available precursors.

The chemical synthesis of estradiol has been  
described (Eder, V. et al., Ber 109, 2948 (1976);  
Oppolzer, D.A. and Roberts, D.A. *Helv. Chim. Acta.* 63,  
1703, (1980)). Synthetic methods for making seven-  
15 membered rings in multi-cyclic compounds are known  
(Nakamuru, T. et al. *Chem. Pharm. Bull.* 10, 281 (1962);  
Sunagawa, G. et al. *Chem. Pharm. Bull.* 9, 81 (1961); Van  
TameLEN, E. E. et al. *Tetrahedron* 14, 8-34 (1961); Evans,  
D. E. et al. *JACS* 103, 5813 (1981)). Those skilled in  
20 the art will appreciate that the chemical synthesis of  
estradiol can be modified to include 7-membered rings by  
making appropriate changes to the starting materials, so  
that ring closure yields seven-membered rings. Estradiol  
or estradiol derivatives can be modified to include  
25 appropriate chemical side groups according to the  
invention by known chemical methods (*The Merck Index*,  
11th Ed., Merck & Co., Inc., Rahway, NJ USA (1989), pp.  
583-584).

Administration

30 The compositions described above can be provided  
as physiologically acceptable formulations using known  
techniques, and these formulations can be administered by  
standard routes. In general, the combinations may be  
administered by the topical, oral, rectal or parenteral  
35 (e.g., intravenous, subcutaneous or intramuscular) rout .

- 9 -

- A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.
- 10 The tablets may optionally be coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

20 Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the

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Example 1:

Figure 1 illustrates the inhibition of tubulin polymerization by 2-methoxyestradiol.

A. Each reaction mixture (all concentrations refer to the final reaction volume of 0.25 ml) contained 1.0 M monosodium glutamate (pH 6.6), 1.0 mg/ml (10  $\mu$ M) tubulin, 1.0 mM MGCl<sub>2</sub>, 4% (v/v) dimethylsulfoxide, and either 0 (curve 1), 20  $\mu$ M (curve 2), 40  $\mu$ M (curve 3), or 75  $\mu$ M (curve 4) 2-methoxyestradiol. The 0.24 ml reaction mixtures were incubated for 15 min at 37°C and chilled on ice. After addition of 10  $\mu$ l of 2.5 mM GTP the reaction mixtures were transferred to cuvettes held at 0°C, and baselines were established. At time zero the temperature controller was set at 37°C. At the times indicated by the vertical dashed lines the temperature controller was set at the indicated temperatures.

B. Each reaction mixture contained 0.8 M monosodium glutamate (pH 6.6), 1.2 mg/ml (12  $\mu$ M) tubulin, 4% (v/v) dimethylsulfoxide, and either 0 (curve 1), 1.0  $\mu$ M (curve 2), 2.0  $\mu$ M (curve 3), 3.0  $\mu$ M (curve 4), or 4.0  $\mu$ M (curve 5) 2-methoxyestradiol. The 0.24 ml reaction mixtures were incubated for 15 min at 26°C and chilled on ice. After addition of 10  $\mu$ l of 10 mM GTP the reaction mixtures were transferred to cuvettes held at 0°C, and baselines were established. At time zero the temperature controller was set at 26°C. At the time indicated by vertical dashed line the temperature controller was set at 0°C.

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Table 1

<u>Estrogenic Compound</u>	<u>IC<sub>50</sub> (μM ± S.D.)</u>
2-Methoxyestradiol	1.9 ± 0.2
Diethylstilbestrol	2.4 ± 0.4
5 2-Bromoestradiol	4.5 ± 0.6
2-Methoxyestrone	8.8 ± 1
17-Ethynylestradiol	10.0 ± 2
2-Fluoroestradiol	27.0 ± 6
Estradiol	30.0 ± 6
10 Estrone	> 40
2-Methoxy-17-ethynylestradiol	> 40
Estriol	> 40
2-Methoxyestriol	> 40
Estradiol-3-O-methyl ether	> 40
15 2-Methoxyestradiol-3-O-methyl ether	> 40
4-Methoxyestradiol	> 40
4-Methoxyestradiol-3-O-methyl ether	> 40

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<u>Plant Products</u>	<u>IC<sub>50</sub> (μM ± S.D.)</u>
20 Colchicine	0.80 ± 0.07
Podophyllotoxin	0.46 ± 0.02
Combretastatin A-4	0.53 ± 0.05
Dihydrocombretastatin A-4	0.63 ± 0.03

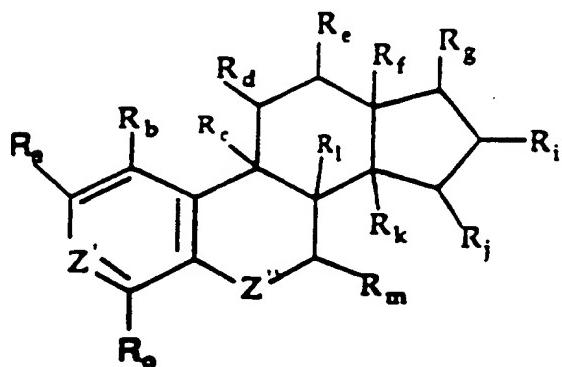
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- 25 IC<sub>50</sub> values are defined as the concentration of an estradiol derivative required to inhibit tubulin polymerization by 50%. IC<sub>50</sub> values were obtained in at least two independent experiments for non-inhibitory agents (IC<sub>50</sub> > 40 μM) and at least three independent experiments for inhibitory compounds. IC<sub>50</sub> values were obtained graphically, and average values are presented. S.D., standard deviation.

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Claims

1. A method of making a medicament which is capable of inhibiting abnormal cell mitosis, said medicament comprising, in a pharmaceutically acceptable carrier, a cell mitosis-inhibiting compound of the formula:



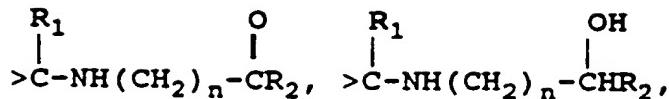
wherein:

I.  $R_a-R_o$  are defined as follows:

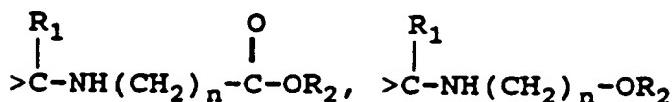
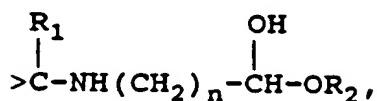
- 10           A) each  $R_a$ ,  $R_b$ ,  $R_c$ ,  $R_d$ ,  $R_e$ ,  $R_f$ ,  $R_i$ ,  $R_j$ ,  $R_k$ ,  $R_l$ ,  $R_m$ ,  $R_o$ , independently is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ , or  $-I$ ; and  $R_g$  is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ ,  $-I$ , or  $-C\equiv CH$ ;

15 or

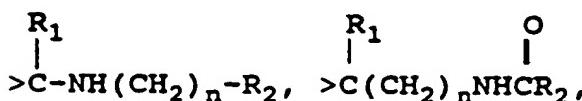
- 17 -



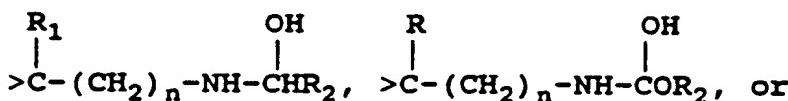
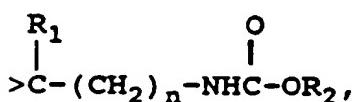
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15



20



or

B)  $\text{Z}''$  is  $-\text{Y}-\text{CH}-$  or  $-\text{CH}-\text{Y}-$  where  $\text{R}_p$

25

$\begin{array}{c} \text{R}_p \\ | \\ \text{R}_p \end{array}$   $\begin{array}{c} \text{R}_p \\ | \\ \text{R}_p \end{array}$   
is  $-\text{R}_1, -\text{OR}_1, -\text{SR}_1, -\text{F}, -\text{NHR}_2, -\text{Br}$  or  $-\text{I}$ ;

and

IV. provided that when each  $\text{R}_b, \text{R}_c, \text{R}_d, \text{R}_e, \text{R}_i, \text{R}_j, \text{R}_k,$   
 $\text{R}_l, \text{R}_m$  and  $\text{R}_o$  is H;

30

$\text{R}_f$  is  $-\text{CH}_3$ ;

$\text{R}_g$  is  $-\text{OH}$ ;

$\text{Z}'$  is  $>\text{COH}$ ; and

$\text{Z}''$  is  $>\text{CH}_2$ ;

then  $\text{R}_a$  is not H;

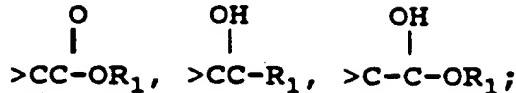
35 where, in each formula set forth above, each  $\text{R}_1$  and  $\text{R}_2$  independently is H, or substituted or unsubstituted alkyl, alkenyl or alkynyl group of 1-6 carbons.

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II. Z' is defined as follows:

5

A) Z' is X, where X is  $>\text{COR}_1$ ,  $>\text{CC}-\text{R}_1$ ,



or

10

B) Z' is  $=\text{C}-\text{X}'-$  or  $-\text{X}'-\text{C}=$ , where  $\text{R}_n$

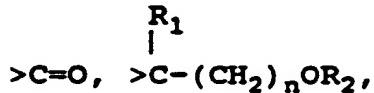
$\begin{array}{c} | \\ \text{R}_n \end{array}$   $\begin{array}{c} | \\ \text{R}_n \end{array}$   
is  $-\text{R}_1$ ,  $-\text{OR}_1$ ,  $-\text{SR}_1$ ,  $-\text{F}$ ,  $-\text{NHR}_2$ ,  $-\text{Br}$  or  
 $-\text{I}$ , and X' is X, as defined above;  
or X' is also  $>\text{C}=\text{O}$ ;

15 and

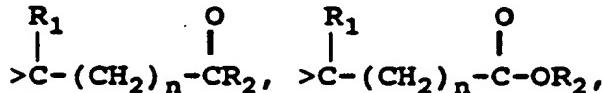
III. Z" is defined as follows:

20

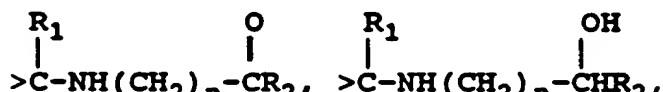
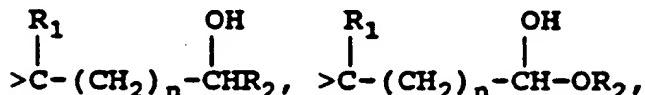
A) Z" is Y, where Y is  $-\text{O}-$ ,  $-\text{N}-$ ,  $>\text{CHR}_1$ ,



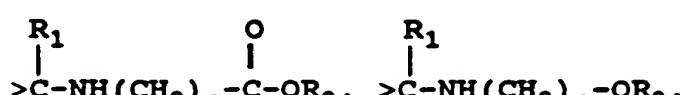
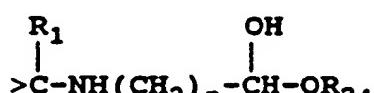
25



30



35



- 21 -

wherein:

I.  $R_a$ - $R_o$  are defined as follows:

A) each  $R_a$ ,  $R_b$ ,  $R_c$ ,  $R_d$ ,  $R_e$ ,  $R_f$ ,  $R_i$ ,  $R_j$ ,  $R_k$ ,  
 5  $R_l$ ,  $R_m$ ,  $R_o$  independently is  $-R_1$ ,  $-OR_1$ ,  
 $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ , or  $-I$ ; and  
 $R_g$  is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  
 $-Br$ ,  $-I$  or  $-C\equiv CH$ ;

or

B) each  $R_a$ ,  $R_b$ ,  $R_c$ ,  $R_f$ ,  $R_k$ ,  $R_l$ ,  
 10  $R_i$ ,  $R_m$ ,  $R_o$  independently is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  
 $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ , or  $-I$ ; and each  
 $R_d$ ,  $R_e$ ,  $R_j$ ,  $R_m$ ,  $R_o$  independently is  
 15  $=O$ ,  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  
 $-Br$ , or  $-I$ ; and  $R_g$  is  $=O$ ,  $-R_1$ ,  $-OR_1$ ,  
 $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  
 $-Br$ ,  $-I$  or  $-C\equiv CH$ ;

and

II. Z is defined as follows:

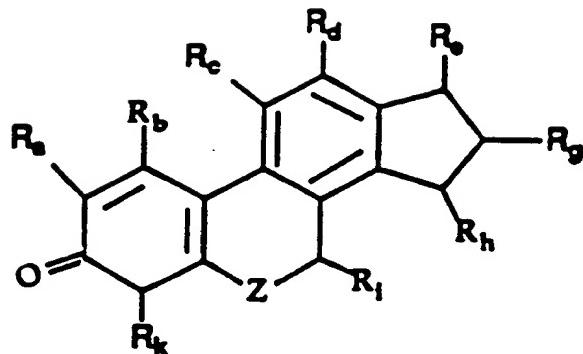
A) Z is Y, where Y is  $-O-$ ,  $-N-$ ,  $>CHR_1$ ,  
 20  $\begin{array}{c} R_1 \\ | \\ >C=O \end{array}$ ,  $>C-(CH_2)_nOR_2$ ,

$\begin{array}{cccc} R_1 & O & R_1 & O \\ | & | & | & | \\ >C-(CH_2)_n-CR_2, & >C-(CH_2)_n-C-OR_2, & & \\ & & & \end{array}$

$\begin{array}{cc} R_1 & OH \\ | & | \\ >C-(CH_2)_n-CHR_2, & \end{array}$

$\begin{array}{cc} R_1 & OH \\ | & | \\ >C-(CH_2)_n-CH-OR_2, & \end{array}$

- 23 -



wherein:

I.  $R_a-R_k$  are defined as follows:

- A) each  $R_a, R_b, R_c, R_d, R_g, R_h, R_i, R_k$  independently is  $-R_1, -OR_1, -OCOR_1, -SR_1, -F, -NHR_1, -Br$ , or  $-I$ ; and  $R_e$  is  $-R_1, -OR_1, -OCOR_1, -SR_1, -F, -NHR_1, -Br, -I$  or  $-C\equiv CH$ ;

or

- B) each  $R_a, R_b, R_c, R_d$ , independently is  $-R_1, -OR_1, -OCOR_1, -SR_1, -F, -NHR_1, -Br$ , or  $-I$  and each  $R_g, R_h, R_i, R_k$  independently is  $=O, -R_1, -OR_1, -OCOR_1, -SR_1, -F, -NHR_1, -Br$  or  $-I$ ; and  $R_e$  is  $=O, -R_1, -OR_1, -OCOR_1, -SR_1, -F, -NHR_1, -Br, -I$  or  $-C\equiv CH$ ;

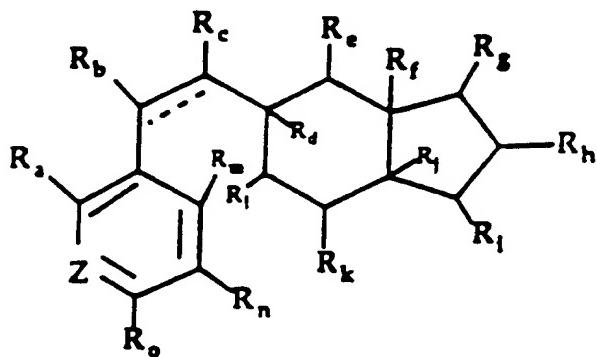
and

II.  $Z$  is defined as follows:

- 25 -

where, in each formula set forth above, each R<sub>1</sub> and R<sub>2</sub> independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynyl group of 1-6 carbons.

5. A method of making a medicament which is capable of inhibiting abnormal cell mitosis, said medicament comprising, in a pharmaceutically acceptable carrier, a cell mitosis-inhibiting compound of the formula:



10 wherein:

I. R<sub>a</sub>-R<sub>o</sub> are defined as follows:

- A) each R<sub>a</sub>, R<sub>b</sub>, R<sub>c</sub>, R<sub>d</sub>, R<sub>e</sub>, R<sub>f</sub>, R<sub>g</sub>, R<sub>h</sub>, R<sub>j</sub>, R<sub>k</sub>, R<sub>l</sub>, R<sub>m</sub>, R<sub>n</sub>, R<sub>o</sub> independently is -R<sub>1</sub>, -OR<sub>1</sub>, -OCOR<sub>1</sub>, -SR<sub>1</sub>, -F, -NHR<sub>2</sub>, -Br, or -I; and R<sub>i</sub> is -R<sub>1</sub>, -OR<sub>1</sub>, -OCOR<sub>1</sub>, -SR<sub>1</sub>, -F, -NHR<sub>2</sub>, -Br, -I or -C≡CH;

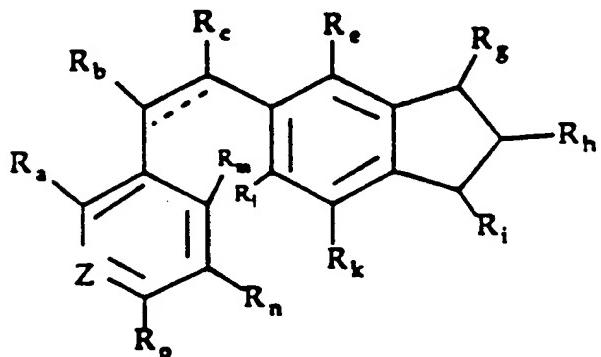
15

or

- B) each R<sub>a</sub>, R<sub>d</sub>, R<sub>f</sub>, R<sub>j</sub>, R<sub>m</sub>, R<sub>n</sub>, R<sub>o</sub> independently is -R<sub>1</sub>, -OR<sub>1</sub>, -OCR<sub>1</sub>, -SR<sub>1</sub>,

- 27 -

6. A method of making a medicament which is capable of inhibiting abnormal cell mitosis, said medicament comprising, in a pharmaceutically acceptable carrier, a cell mitosis-inhibiting compound of the  
5 formula:



wherein:

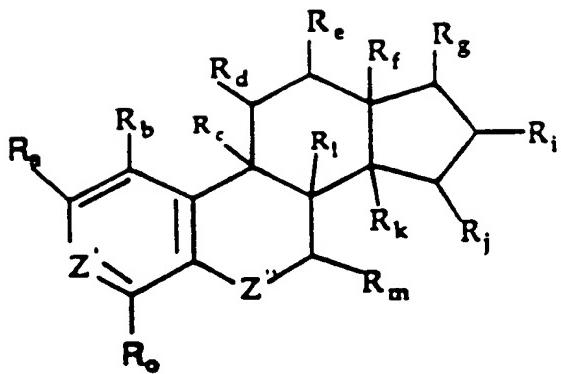
I.  $R_a-R_o$  are defined as follows:

- A) each  $R_a$ ,  $R_b$ ,  $R_c$ ,  $R_e$ ,  $R_g$ ,  $R_h$ ,  $R_k$ ,  $R_l$ ,  $R_m$ ,  
10  $R_n$ ,  $R_o$  independently is  $-R_1$ ,  $-OR_1$ ,  
 $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ , or  $-I$ ; and  
 $R_1$  is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  
 $-Br$ ,  $-I$  or  $-C\equiv CH$ ;

or

- B) each  $R_a$ ,  $R_e$ ,  $R_l$ ,  $R_m$ ,  $R_n$ ,  $R_o$  independently  
15 is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  
 $-Br$ ,  $-I$  and each  $R_b$ ,  $R_c$ ,  $R_g$ ,  $R_h$  is  $=O$ ,

- 29 -



wherein:

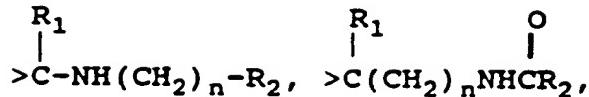
I.  $R_a$ - $R_o$  are defined as follows:

(A) each  $R_a$ ,  $R_b$ ,  $R_c$ ,  $R_d$ ,  $R_e$ ,  $R_f$ ,  $R_i$ ,  $R_j$ ,  $R_k$ ,  $R_l$ ,  $R_m$ ,  $R_o$ , independently is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ , or  $-I$ ; and  $R_g$  is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ ,  $-I$  or  $-C\equiv CH$ ;

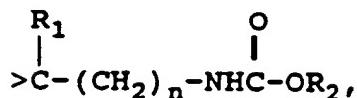
or

(B) each  $R_a$ ,  $R_b$ ,  $R_c$ ,  $R_f$ ,  $R_k$ ,  $R_l$ ,  $R_o$ , is  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ , or  $-I$ ; and each  $R_d$ ,  $R_e$ ,  $R_i$ ,  $R_j$ ,  $R_m$ , independently is  $=O$ ,  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$  or  $-I$ ; and  $R_g$  is  $=O$ ,  $-R_1$ ,  $-OR_1$ ,  $-OCOR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$ ,  $-I$  or  $-C\equiv CH$ ;

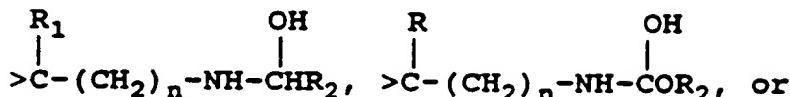
- 31 -



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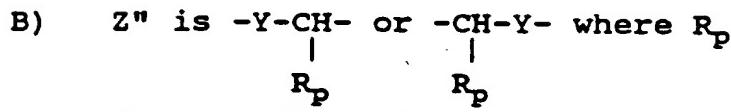


10



or

15



is  $-\text{R}_1, -\text{OR}_1, -\text{SR}_1, -\text{F}, -\text{NHR}_2, -\text{Br}$  or  $-\text{I}$ ;  
provided that when:

20

3) each  $\text{R}_b, \text{R}_c, \text{R}_d, \text{R}_e, \text{R}_j, \text{R}_k, \text{R}_l, \text{R}_m$ , is  
 $-\text{H}$ ;

$\text{R}_f$  is  $-\text{CH}_3$ ;

25

$\text{R}_g$  is  $-\text{OH}, -\text{OCCH}_3$ ;

$\text{R}_i$  is  $-\text{H}, -\text{OH}$ , or  $=\text{O}$ ;

$\text{R}_o$  is  $-\text{H}$  or  $-\text{Br}$ ;

$\text{Z}'$  is  $>\text{COH}$ ; and

$\text{Z}''$  is  $>\text{CH}_2$  or  $-\text{OH}$ ; then

$\text{R}_a$  is not  $-\text{F}, -\text{Br}, -\text{OH}$  or  $-\text{H}$ ;

30 and

4) each  $\text{R}_b, \text{R}_c, \text{R}_d, \text{R}_e, \text{R}_i, \text{R}_j, \text{R}_k, \text{R}_l,$   
 $\text{R}_m$ , is  $-\text{H}$ ;

$\text{R}_f$  is  $-\text{CH}_3$ ;

$\text{R}_g$  is  $-\text{OH}$ ; and

35

$\text{Z}''$  is  $>\text{CH}_2$ ; then

- 33 -

wherein:

I.  $R_a-R_k$  are defined as follows:

A) each  $R_a, R_b, R_c, R_d, R_g, R_h, R_i, R_k$  independently is  $-R_1, -OR_1, -OCOR_1,$   
 5  $-SR_1, -F, -NHR_2, -Br, \text{ or } -I;$  and  $R_e$  is  
 $-R_1, -OR_1, -OCOR_1, -SR_1, -F, -NHR_2, -Br,$   
 $-I \text{ or } -C\equiv CH;$

or

B) each  $R_a, R_b, R_c, R_d, R_k$  is  $-R_1, -OR_1,$   
 10  $-OCOR_1, -SR_1, -F, -NHR_2, -Br, \text{ or } -I;$  and  
 each  $R_g, R_h, R_i$ , independently is  $=O,$   
 $-R_1, -OR_1, -OCOR_1, -SR_1, -F, -Br, \text{ or } -I;$   
 and  $R_e$  is  $=O, -R_1, -OR_1, -OCOR_1, -SR_1,$   
 $-F, -Br, -I \text{ or } -C\equiv CH;$

15 and

I.  $Z'$  is defined as follows:

A)  $Z'$  is  $X$ , where  $X$  is  $>COR_1, >C_2C-R_1,$   
 20  $>C_2C-OR_1, >CC-R_1, >C-C-OR_1;$

or

B)  $Z'$  is  $=C-X'-$  or  $-X'-C=$ , where  $R_n$   
 25  $\begin{array}{c} | \\ O \\ | \\ R_n \end{array} \quad \begin{array}{c} OH \\ | \\ O \\ | \\ R_n \end{array}$   
 $is -R_1, -OR_1, -SR_1, -F, -NHR_2, -Br \text{ or }$   
 $-I, \text{ and } X' \text{ is } X, \text{ as defined above;}$   
 $or X' \text{ is also } >C=O;$

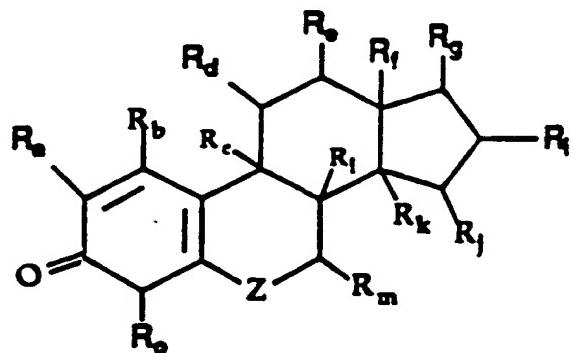
30 and

II.  $Z''$  is defined as follows:

- 35 -

where, in each formula set forth above, each R<sub>1</sub> and R<sub>2</sub> independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynyl group of 1-6 carbons.

9. A compound of the general formula below, said 5 compound being a cell-mitosis-inhibiting compound:



wherein:

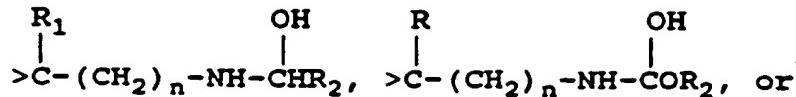
I. R<sub>a</sub>-R<sub>o</sub> are defined as follows:

- A) each R<sub>a</sub>, R<sub>b</sub>, R<sub>c</sub>, R<sub>d</sub>, R<sub>e</sub>, R<sub>f</sub>, R<sub>i</sub>, R<sub>j</sub>, R<sub>k</sub>, R<sub>l</sub>, R<sub>m</sub>, R<sub>o</sub> independently is -R<sub>1</sub>, -OR<sub>1</sub>, -OCOR<sub>1</sub>, -SR<sub>1</sub>, -F, -NHR<sub>2</sub>, -Br, or -I; and R<sub>g</sub> is -R<sub>1</sub>, -OR<sub>1</sub>, -OCOR<sub>1</sub>, -SR<sub>1</sub>, -F, -NHR<sub>2</sub>, -Br, -I or -C≡CH;

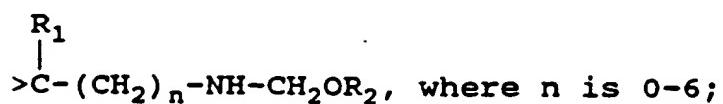
or

- B) each R<sub>a</sub>, R<sub>b</sub>, R<sub>c</sub>, R<sub>f</sub>, R<sub>k</sub>, R<sub>l</sub>, independently is -R<sub>1</sub>, -OR<sub>1</sub>, -OCOR<sub>1</sub>, -SR<sub>1</sub>, -F, -NHR<sub>2</sub>, -Br, or -I; and each R<sub>d</sub>, R<sub>e</sub>, R<sub>i</sub>, R<sub>j</sub>, R<sub>m</sub>, R independently is =O, -R<sub>1</sub>, -OR<sub>1</sub>, -OCOR<sub>1</sub>,

- 37 -



5



or

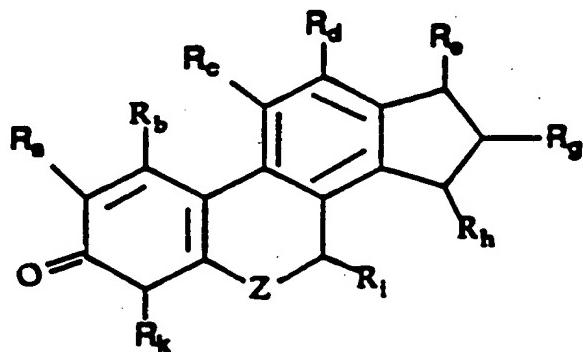
B) Z is  $\begin{array}{c} \text{Y}-\text{CH}- \\ | \\ \text{R}_n \end{array}$  or  $\begin{array}{c} -\text{CH}-\text{Y}- \\ | \\ \text{R}_n \end{array}$ , where  $\text{R}_n$

10

is  $-\text{R}_1$ ,  $-\text{OR}_1$ ,  $-\text{SR}_1$ ,  $-\text{F}$ ,  $-\text{NHR}_2$ ,  $-\text{Br}$  or  
-I;

where, in each formula set forth above, each  $\text{R}_1$  and  $\text{R}_2$   
independently is -H, or substituted or unsubstituted  
15 alkyl, alkenyl or alkynyl group of 1-6 carbons.

10. A compound of the general formula below, said  
compound being a cell-mitosis-inhibiting compound:

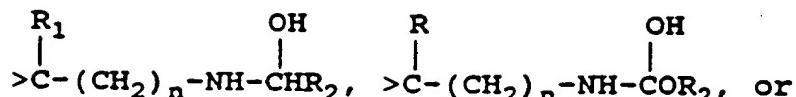


wherein:

20 I.  $\text{R}_a-\text{R}_k$  are defined as follows:

A) each  $\text{R}_a$ ,  $\text{R}_b$ ,  $\text{R}_c$ ,  $\text{R}_d$ ,  $\text{R}_e$ ,  $\text{R}_f$ ,  $\text{R}_g$ ,  $\text{R}_h$ ,  $\text{R}_i$ ,  $\text{R}_j$ ,  $\text{R}_k$   
independently is  $-\text{R}_1$ ,  $-\text{OR}_1$ ,  $-\text{OCOR}_1$ ,  
 $-\text{SR}_1$ ,

- 39 -

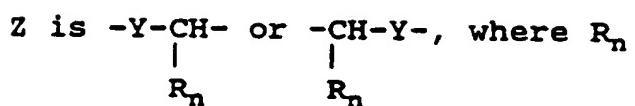


5



or

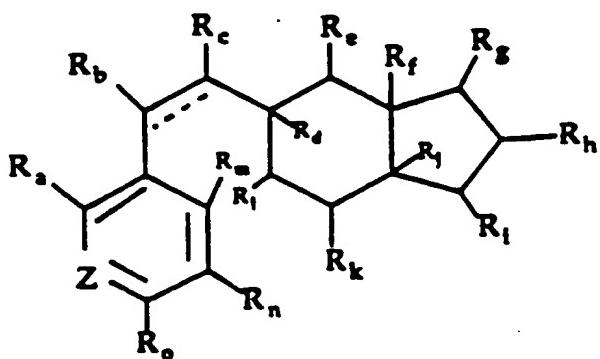
10



is  $-\text{R}_1$ ,  $-\text{OR}_1$ ,  $-\text{SR}_1$ ,  $-\text{F}$ ,  
 $-\text{NHR}_2$ ,  $-\text{Br}$  or  $-\text{I}$ ;

where, in each formula set forth above, each  $\text{R}_1$  and  $\text{R}_2$   
 independently is  $-\text{H}$ , or substituted or unsubstituted  
 15 alkyl, alkenyl or alkynyl group of 1-6 carbons.

11. A compound of the general formula below, said compound being a cell-mitosis-inhibiting compound:



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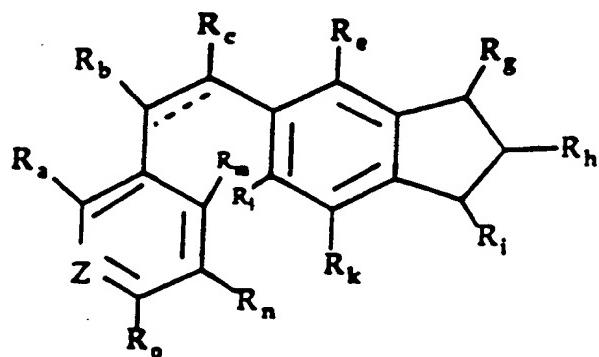
Z is  $=C-X'-$  or  $-X'-C=$ , where R<sub>p</sub>  
 |                   |  
 R<sub>p</sub>              R<sub>p</sub>

5

is  $-R_1$ ,  $-OR_1$ ,  $-SR_1$ ,  $-F$ ,  $-NHR_2$ ,  $-Br$  or  
 -I; and X' is X, as defined above;  
 or X' is  $>C=O$ ;

where, in each formula set forth above, each R<sub>1</sub> and R<sub>2</sub>  
 independently is -H, or substituted or unsubstituted  
 alkyl, alkenyl or alkynyl group of 1-6 carbons; and the  
 10 bond indicated by C $\bullet\bullet$ C is absent or, in combination with  
 the C-C bond is the unit HC=CH.

12. A compound of the general formula below, said  
 compound being a cell-mitosis-inhibiting compound:



15 wherein:

I. R<sub>a</sub>-R<sub>o</sub> are defined as follows:

- 43 -

where, in each formula set forth above, each R<sub>1</sub> and R<sub>2</sub> independently is -H, or substituted or unsubstituted alkyl, alkenyl or alkynyl group of 1-6 carbons; and the bond indicated by C•••C is absent or, in combination with 5 the C-C bond is the unit HC=CH.

13. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-methoxyestradiol.

14. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-fluoroestradiol.

15. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-bromoestradiol.

16. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 2-methoxyestrone.

17. The method of claim 1, wherein said cell-mitosis-inhibiting compound is 17-ethynylestradiol.

18. The method of claims 1 or 2 wherein said compound is further characterized in that

A) Z' is =C-X'- or -X'-C=; and  
| |  
R<sub>n</sub> R<sub>n</sub>

Z" is -Y-CH- or -CH-Y-; or  
| |  
R<sub>p</sub> R<sub>p</sub>

B) Z' is X; and Z" is -Y-CH- or -CH-Y-; or  
| |  
R<sub>p</sub> R<sub>p</sub>

C) Z' is =C-X'- or -X'-C=; and Z" is Y.  
| |  
R<sub>n</sub> R<sub>n</sub>

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25. The compound of any one of claims 7-12,  
wherein at least one of  $R_a$ - $R_p$  is  $-OCH_3$ .

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US94/08767

**A. CLASSIFICATION OF SUBJECT MATTER:**  
IPC (6):

A61K 31/56; C07J 41/00, 31/00, 13/00, 9/00, 5/00, 7/00, 3/00, 1/00.

**A. CLASSIFICATION OF SUBJECT MATTER:**  
US CL :

514/177, 178, 179, 182; 552/516, 522, 523, 524, 525, 535, 536, 540, 541, 542, 543, 544, 548, 549, 550, 551, 552, 553, 554, 555, 557, 558, 559, 560, 562, 563, 564, 565, 566, 567, 569, 571, 572, 573, 575, 582, 583, 584, 585, 599, 603, 604, 605, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 623, 624, 625, 626, 627, 628, 629, 642, 643, 644, 646, 647, 650, 651, 652.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

Group I, claims 1, 3, 7, 9, and 24, each in part, and claims 13-17; directed to a method of making a medicament compound in which the A ring is aromatic and said compound.

Group II, claims 1, 7, 9, 18, 21, each in part, and 25, directed to a method of making a medicament in which the A ring is aromatic and Z<sup>\*</sup> is Y and Y is O.

Group III, claims 1, 7, 9, 18, 21, each in part, and 25, as in Group II, except that Y is N.

Group IV, claims 1, 3, 7, 18, 21, each in part, and 25, as in Group II, except that the A ring is aromatic and contains 7 carbons and the B ring contains 6 carbon atoms.

Group V, claims 1, 3, 7, 9, 18, 21, each in part, and 25, as in Group IV except that the B ring contains 7 carbon atoms.

Group VI, claims 1 and 18, each in part, in which the A ring contains 6 carbon atoms and the B ring contains 7 carbon atoms.

Group VII, claims 1, 3, 7, 18, 25, each in part, and 25, in which A ring is 7 carbon aromatic and the B ring contains carbons, Z<sup>\*</sup> is Y and Y is O.

Group VIII, claims 1, 3, 7, 18, 21, each in part, and 25, as in Group VII in which Y is N.

Group IX, claims 2, 4, 8, 10, 18, 21, each in part, and 25, directed to a method of making a medicament in which the A and C rings are each aromatic.

Group X, claims 2, 8, 10, 18, 21, each in part, and 25, directed to a method of making a medicament compound as in Group IX in which the Z<sup>\*</sup> is Y and Y is O.

Group XI, claims 2, 8, 10, 18, 21, each in part, and 25, as in Group IX in which Y is N.

Group XII, claims 2, 8, 18, 21, each in part, and 25, as in Group IX in which the A ring contains 7 carbon atoms.

Group XIII, claims 2 and 18, each in part, as in Group VIII, in which the B ring contains 7 carbon atoms.

Group XIV, claims 2, 18, 25, each in part, and 25 as in Group XII, in which the B ring contains 7 atoms.

Group XV, claims 2, 8, 10, 18, each in part, and 25 in which the B ring has 7 carbons and Y is O.

Group XVI, claims 2, 8, 18, each in part, and 25 in which the B ring has 7 carbons and Y is N.

Group XVII, claims 3 in part, 19, and 24, directed to a method of making a medicament in which there is a keto group at C3, the A ring is aromatic and the B ring contains 6 carbon atoms.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/08767

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/177, 178, 179, 182; 552/558, 614, 617, 625, 627

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS online

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Steroid Biochem., Vol. 32, No. 6, issued 1989, J. Seegers et al., "The Cytotoxic Effects of Estradiol-17beta, catecholestradiols and methoxyestradiols on dividing MCF-7 and HeLa cells" pages 797-809, see entire article.	1, 7, 13 -----
--		
Y		14, 15, 16, 17, 24
X	Chemical Abstracts, Vol. 105, issued 1986, W.J. Wheeler et al., "Mitotic inhibition and aneuploidy induction by naturally occurring and synthetic estrogens in Chinese hamster cells in vitro", see abstract no. 54822, Mutat. Res., 171(1), 1986, 31-41.	1, 17 -----
--		
Y		13, 14, 15, 16, 24

Further documents are listed in the continuation of Box C.

See patent family annex.

• Special categories of cited documents:	
•A• document defining the general state of the art which is not considered to be of particular relevance	•T• later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
•E• earlier document published on or after the international filing date	•X• document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
•L• document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	•Y• document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
•O• document referring to an oral disclosure, use, exhibition or other means	•&• document member of the same patent family
•P• document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search	Date of mailing of the international search report
20 OCTOBER 1994	10 NOV 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer
Faxsimile No. (703) 305-3230	REBECCA COOK jd Telephone No. (703) 308-1235 <i>Rebecca Cook</i>

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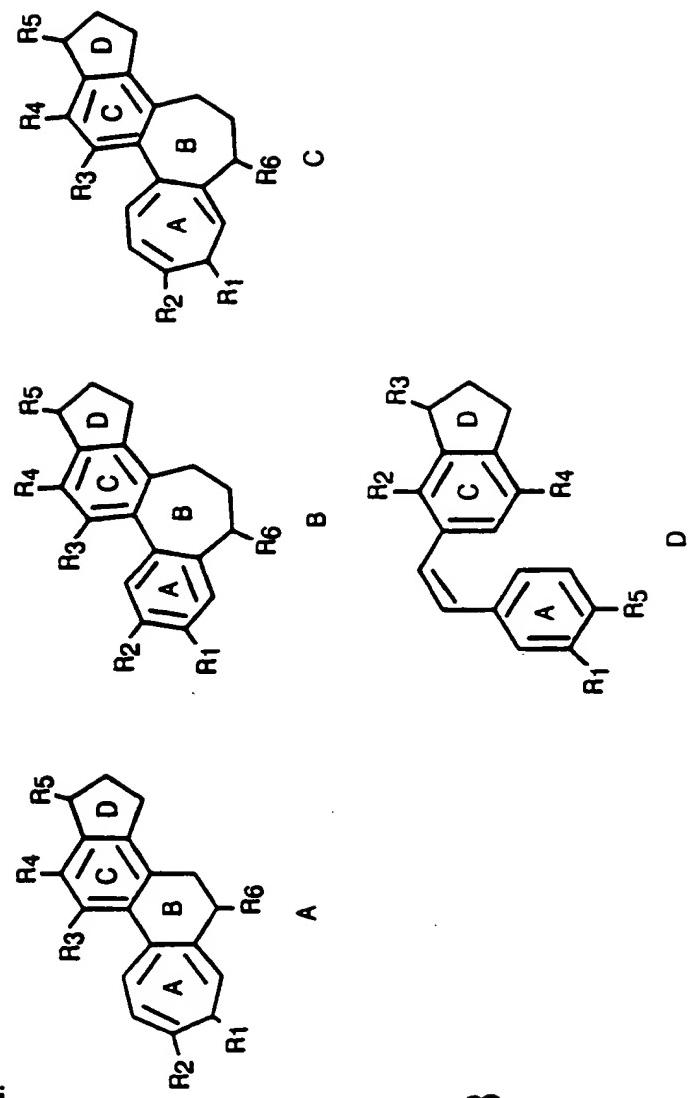
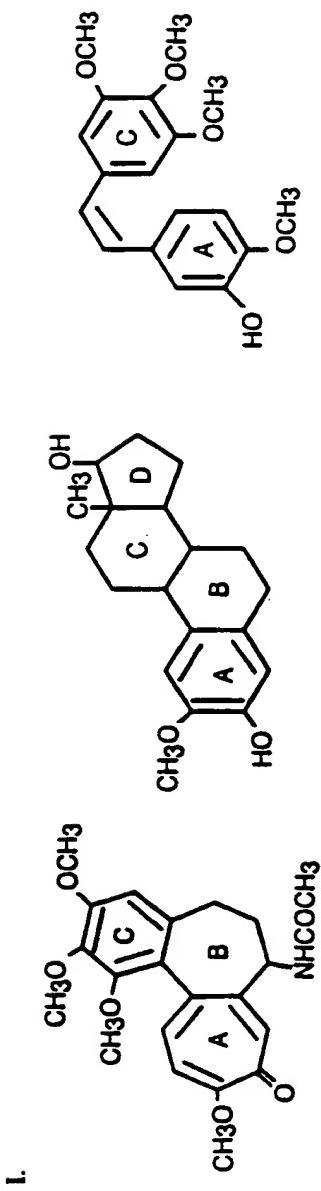
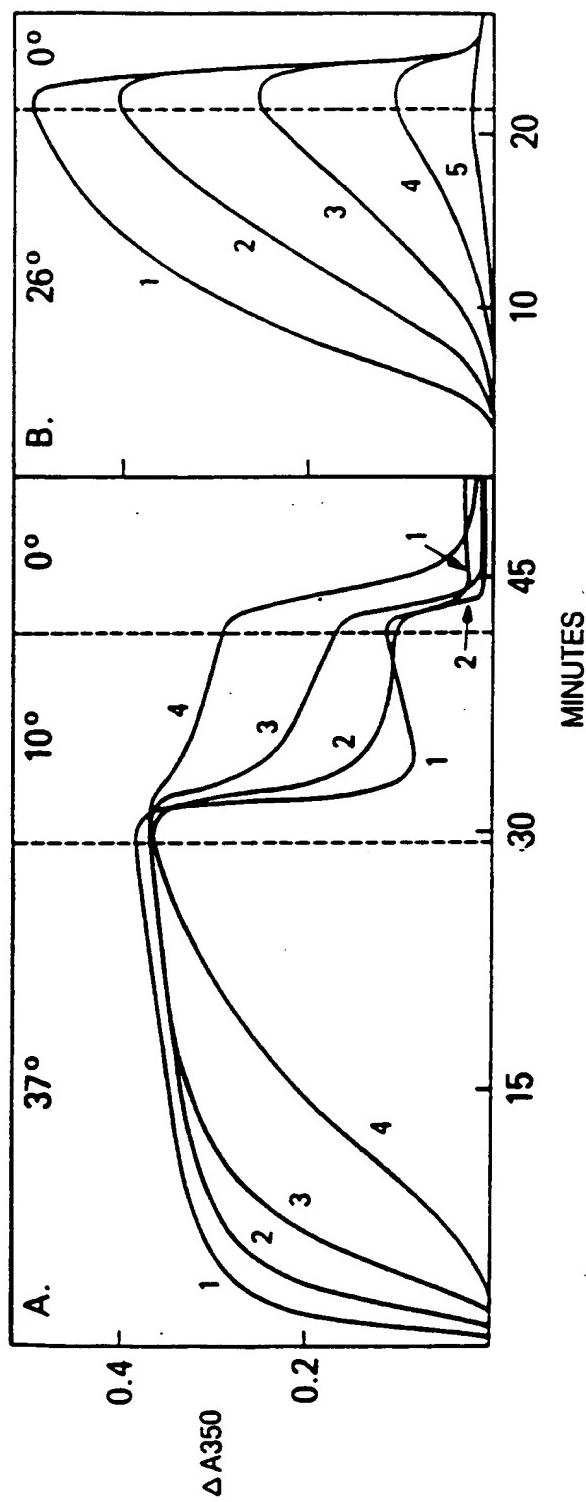


FIG. 3

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FIG. 1



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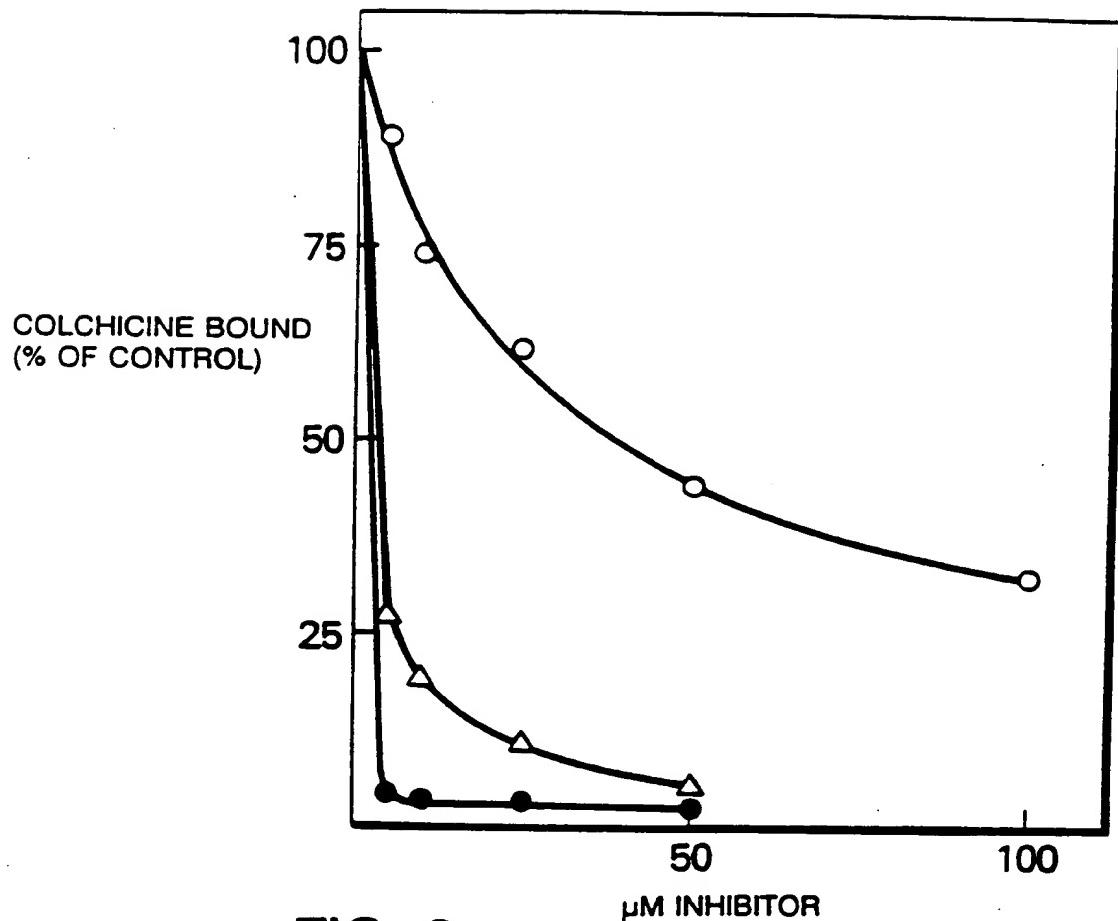


FIG. 2